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Short Communication

Regeneration of plantlets from leaf and internode explants of *Phyllanthus amarus* Schum. and Thonn.

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A procedure is outlined for indirect organogenesis of *Phyllanthus amarus* using leaf bits and internodes. Profuse callusing of leaf discs and internodes were obtained on Murashige and Skoog's basal medium supplemented with NAA and 2,4-D. The callus thus obtained was repeatedly sub-cultured at 3 weekly intervals for four cycles. High frequency of callus proliferation was obtained when the callus was sub-cultured on MS medium supplemented with BAP (1.0 mgl^{-1}) and glycine (50.0 mgl^{-1}). Complete plantlets were obtained when the callus was sub-cultured on MS medium supplemented with BAP (2.0 mgl^{-1}) and GA_3 (0.5 mgl^{-1}). Rooting (87.09%) of the shoots was best achieved on half strength MS medium supplemented with IBA (0.5 mgl^{-1}) and IAA (0.5 mgl^{-1}). Regenerated plants were successfully transferred to soil after acclimatizing them in the plant growth chamber.

Key words: *Phyllanthus amarus*, callus, internode, leaf, micropropagation.

INTRODUCTION

Phyllanthus amarus Schum. and Thonn. (Euphorbiaceae) has been traditionally used in the treatment of a variety of ailments including hepatic disorders (Kirtikar and Basu, 1993; Nadkarni, 1976). The plant is being used as one of the important ingredients in many indigenous poly herbal formulations and other ayurvedic preparations. It is used in stomach troubles such as dyspepsia, colic, diarrhea and dysentery and also for dropsy and diseases of urino genital system. Fresh root is a remedy for jaundice. Leaves are stomachic. Milky juice is used as application to offensive to sore. Powdered leaves and roots are pulverized and made into poultice with rice-water and used to lessen oedematous swellings and ulcers. Leaves are a popular remedy against fever and infusion of young shoots is given in dysentery.

The conventional method of propagation of these species is through seeds. However, poor germination potential restricts their multiplication. Micropropagation technique offers an alternative method for cloning these plants (Unander, 1991; Santos et al., 1994). This research describes the micro-propagation of *P. amarus* from leaf discs and internodes and successful establishment of plantlets in soil.

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MATERIALS AND METHODS

Two month old plants of *P. amarus* grown and maintained in the green house, Medicinal Plant Conservatory of the Botanical Garden, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India were used as the source of explants. Excised leaf bits (0.5 - 1.0 cm^2) and internodes (1.0 - 1.5 cm) were initially washed with teepol for 2 min and then under running tap water for 3 min. This was followed by treating with 0.1% mercuric chloride for 2 min. After washing 4 - 5 times with sterile distilled water and inoculated on basal medium consisting of Murashige and Skoog's (1962) salts and vitamins, 3% sucrose and 0.8% agar. Basal medium was supplemented with various concentrations of NAA (0.1 - 0.5 mgl^{-1}) and 2,4-D (1.0 - 5.0 mgl^{-1}) for callus induction. The regeneration medium consisted of MS basal medium supplemented with BAP (1.0 - 5.0 mgl^{-1}) and GA_3 (0.5 mgl^{-1}). Half strength MS medium supplemented with IAA (0.25 - 1.25 mgl^{-1}) and IBA (0.25 - 1.25 mgl^{-1}) for rooting. The medium was buffered to pH 5.8 and dispensed in 25 x 150 mm culture tubes before autoclaving at 121°C for 15 min. All cultures were maintained at 25 ± 2°C, under 16 h photoperiod provided by cool white fluorescent light (35 µEM-2S-1) with 70% relative humidity. Each experiment was performed thrice with total number of 20 inoculated explants per treatment.

After eight weeks of callus initiation, callus clumps were transferred to regeneration medium. Multiple shoots, developed after 30 days after first sub-culture, were dissected out individually for further multiplication and this process was continued repeatedly every 20 days. Finally the cluster shoots developed were transferred to basal medium additionally supplemented with 1.0 mgl^{-1} GA_3 for elongation before transferring to ½ strength MS medium containing IBA/IAA for rooting. The rooted plantlets were transferred to plastic pots containing sand and vermiculite (1:1), the humidity being main-

Table 1. Effect of 2,4-D and NAA on callus induction from leaf bits and internodes of *P. amarus*.

Treatment (Concentration of 2,4-D + NAA) (mgl^{-1})	Internodes				Leaf bits			
	% of forming callus	Relative growth	Callus Index	Days taken for callusing	% of forming callus	Relative growth	Callus Index	Days taken for callusing
T_1 (1.0 + 0.1)	29.97	1.33	40.24	56.33	20.00	1.21	24.23	59.16
T_2 (2.0 + 0.2)	21.66	1.65	37.33	58.16	40.00	1.20	48.90	57.66
T_3 (3.0 + 0.3)	73.33	2.88	211.81	52.00	62.22	2.60	166.72	51.77
T_4 (4.0 + 0.4)	84.99	3.83	325.62	46.66	84.99	3.77	320.90	49.16
T_5 (5.0 + 0.5)	20.25	1.16	23.14	56.83	21.10	1.43	30.14	58.50
Mean	**46.04**	**2.17**	**127.63**	**54.00**	**45.66**	**2.04**	**118.18**	**55.25**
SEd	6.97	0.19	17.97	1.95	5.07	0.37	31.14	0.88
CD (0.05)	15.54	0.42	40.50	4.35	11.31	0.83	69.40	1.98

Table 2. Effect of BAP and GA_3 on multiple shoot induction from leaf bits and internodes of *P. amarus*

Treatment (Concentration of BAP + GA_3) (mgl^{-1})	Survival percentage	Days taken for shooting	Number of shoots per calli	Length of shoot (cm)
T_1 (1.0 + 0.5)	67.81	24.29	4.66	1.41
T_2 (2.0 + 0.5)	82.04	15.35	5.08	1.72
T_3 (3.0 + 0.5)	63.01	28.64	3.98	1.23
T_4 (4.0 + 0.5)	56.90	29.66	3.09	1.23
T_5 (5.0 + 0.5)	52.83	28.99	3.60	1.07
Mean	**64.52**	**25.39**	**4.08**	**1.33**
S.E.D	1.132	0.310	0.318	0.089
CD (0.05)	2.522	0.690	0.709	0.200

tained by covering with plastic bags. The survival percentage was determined after 20 days in pots.

RESULTS AND DISCUSSION

High callusability (325.62) was observed with stem bit explant and low callusability (320.90) was recorded with leaf bit explant at a concentration of 4.0 mgl^{-1} 2,4-D and 0.4 mgl^{-1} NAA (Table 1). Haicour (1974) obtained callus cultures of leaf bit and stem bit of *Phyllanthus urinaria*. Among the explants stem bit have recorded the best response. This was supported by Unander (1991) found that the stem bit was ideal explants for callus induction in *P. amarus*.

The possibility of callus regeneration that could be brought through the manipulation of hormone balance is a known factor (Rossini, 1969). The level of auxin and cytokinin alone or in combination decides the efficiency of callusability and organogenesis (Kohlenbach, 1977). Leaf bit and internode derived callus showed better regeneration capacity of shoots at the effective concentration of cytokinin (2.0 mgl^{-1} BAP) along with GA_3 (0.5 mgl^{-1}) (Table 2). The effective and efficient role played by the BAP in the shoot proliferation of various medicinal crops were emphasized by Harikrishnan et al. (1999) in *Acorus calamus*, Arockia Samy et al. (1999) in *Datura metel*, Al-Wasel (1999) in *Atropa belladonna*, Smitha et al. (2000) in *Plumbago indica*, Selvakumar and Balakumar (2000) in *Acalypha fruiticosa* and Muthuram et al. (2000) in *Scoparia dulsis*.

GA_3 is known to have stimulatory effect on stem elongation in different plants. The same effect was seen in the present study when GA_3 was supplemented to the MS basal media at a lower concentration responded well to elongation (an average length of 1.72 cm). This was supported by the findings of Sitakanta et al. (1996) in *Ocimum sanctum*. However, above the optimal level a negative correlation was found to exhibit between the concentration of GA_3 and the response to shoot elongation.

Leaf bit and internode derived callus showed better regeneration capacity of roots at the effective concentration of auxin (IBA and IAA at 0.5 mgl^{-1}). Further, addition of IAA (1.25 mgl^{-1}) and IAA (1.25 mgl^{-1}) also gave good response to rooting, however the percentage of rooting was only 15 per cent and yellowing of leaves was also observed (Table 3). This might be due to overdose of auxin. The root elongation phase is very sensitive to auxin concentration and will be inhibited by high concentration as reported by Thimmann (1977).

The rooted plantlets were transferred to hardening chamber. Two types of media *viz.*, sand, soil and leaf mould (1:1:1) and sand and vermiculite (1:1) were tried. Considerable extent of *in vitro* establishment was observed with sand, soil and leaf mould (16.66%) and sand

Table 3. Effect of IAA and IBA on rhizogenesis of *P. amarus*.

Treatment (Concentration of IAA + IBA) (mgl^{-1})	Percentage response to rhizogenesis	Days taken for rooting	Number of roots per plant	Length of root (cm)
T_1 (0.25 + 0.25)	50.63	20.21	2.88	1.94
T_2 (0.50 + 0.50)	87.09	15.31	4.69	5.34
T_3 (0.75 + 0.75)	71.18	18.30	3.34	3.34
T_4 (1.00 + 1.00)	68.23	21.20	5.77	4.04
T_5 (1.25 +10.25)	42.30	21.21	2.03	2.25
Mean	**63.89**	**19.25**	**3.74**	**3.83**
SEd	0.896	0.565	0.337	0.176
CD (0.05)	1.997	1.258	0.751	0.393

Table 4. Establishment of *P. amarus* plantlets *in vivo*.

Medium	Number of plantlets transferred	Survival (%)		
		After 1 week	After 2 week	After 3 week
Sand and vermiculite (1:1)	50	93.33	83.00	80.00
Sand, soil and leaf mould (1:1:1)	50	50.00	33.33	16.66

and vermiculite (80.00%). But the growth was luxurious in vermiculite medium. Within 25 days, it was better established and transferred to pot with soil, sand, farm yard manure from where it can be taken for field planting (Table 4).

REFERENCES

Al-Wasel AS (1999). *In vitro* multiplication of *Atropa belladonna* L. using nodal segments. Alexandria J. Agric. Res. 44: 263-274.

Arockiasamy DI, Muthukumar B, Britto SJ (1999). *In vitro* plant regeneration from internodal segments of *Datura metal* L. Adv. In Plant Sci. 12: 227-231.

Haicour R (1974). Comparasion chez *Phyllanthus urinaria* L. de l' activite antibact erienne desd'ecoctions de diverses portions de la plante et al, de cultures de tissue qui en proviennent. CR Seances Acad Sci Paris S'er D 278: 3323 – 3325.

Harikrishnan KN, Moly Hariharan (1999). *In vitro* clonal propagation of sweet flag (*Acorus calamus*) – a medicinal plant. In: Plant tissue culture and biotechnology emerging trends. Jan : 29-31.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tabacco tissue cultures. Physiol. Plant, 15: 437-497

Muthuram G, Brindha P, Lokeswari TS (2000). *In vitro* propagation of *Scoparia dulcis* Linn. A medicinal herb. In: Recent Trends in Spices and Medicinal Plants Research. Amit Krishna De (Eds.). associated publishing co., New Delhi: 100 – 105.

Nadkarni KM (1976). In Indian Materia Medica. Vol.1: 947 – 949, Popular Prakashan, Bombay.

Rossini (1969). One nogevelle method de culture in vitro de cellules parenchymatessus separess de fevilles de *Calystegia sepium* L. CR. Acad. Sci., Paris, In : Plant tissue culture an dits biotechnology application. W.Berg, E. Rainard, M.H. Zenk, (Eds.). Springer Venlarg, Berlin.

Santos ARS, FilhoNiero VCR, Viana AM, Moreno FN, Campos MM, Yunes RR, Calixto JB (1994). Analgesic effects of callus culture extracts from selected species of *Phyllanthus* in mice. J. Pharma Pharmacol. 46: 755-759.

Selvakumar V, Balakumar T (2000). *In vitro* induction of axillary branching in the medicinal plant *Acalypha fruiticosa* Forsk. In: Recent Trends in Spices and Medicinal Plants Resaerch. Amit Krishna De (Eds.). associated publishing co., New Delhi: 96-97

Sitakanta Pattnaik, Pradeep K.Chand (1996). *In vitro* propagation of the medicinal herbs *Ocimum americanum* L.syn. *Ocimum canum* Sims. and *Ocimum sanctum* L. Plant Cell Rep., 15: 846-850

Smitha Chetia and Handique, PJ (2000). High frequency *in vitro* shoot multiplication of *Plumbago indica*, a rare medicinal plant. Curr. Sci. 78: 1187-1188

Thimmann KV (1977). Hormone action in the whole life of plant. University of Massachusetts Press, Amherst.

Unander DW (1991). Callus induction in *Phyllanthus* spp. and inhibition of viral enzymes by callus extracts. Plant Cell Rep. 10: 461-466.